Effect of Formaldehyde on Cellular Proliferation of HEK293 cells

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Abstract—**In order to investigate the influences of formaldehyde with different doses on the cellular proliferation and cell cycle of the HEK293 cells, methyl thiazol tetrazolium (MTT) assay and flow cytometry were applied to measure the cellular proliferation and cell cycle. After the HEK293 cells were treated by low dose of formaldehyde (60μmol/L) for 12 hours, the percentage of phase G1 (28.4%) was decreased 23.24% compared with that of the control group (37.0%), but the percentage of phase S (48.5%) was increased 19.16% compared with that of the control group (40.7%); After the HEK293 cells were treated by the high dose of formaldehyde (240μmol/L) for 12 hours, the percentage of phase G1 (39.9%) was increased 7.8% compared with that of the control group (37.0%), the percentage of phase S (32.8%) was decreased 19.41% compared with that of the control group (40.7%). The results demonstrate that formaldehyde can promote the cellular proliferation of the HEK293 cells at the low dose (60μmol/L) and can inhibit the cellular proliferation of the HEK293 cells at the high dose (240 μmol/L).**

Keywords- formaldehyde, HEK293 cells, cell proliferation, cell cycle

I. INTRODUCTION

Formaldehyde is one of the indoor air pollutants with the characteristics of extensive sources and serious biological toxicities. Previous studies about the formaldehyde mainly focus on the areas of respiratory stimulation, allergy, oxidative damage, inherent toxicity, and immunotoxicity etc. However, the influence of formaldehyde on cell proliferation and cell cycle is rarely reported. Therefore, in this study, the HEK293 cells were chosen as materials to evaluate the effects of different doses of formaldehyde on HEK293 cell proliferation and cell cycle.

II. MATERIALS AND METHODS

A. Reagents and apparatus

10% formalin, MTT, DMSO, PI and RNase A were purchased from Sigma. RPMI1640 and fetal bovine serum (FBS) were purchased from Gibco. All other reagents were of the highest grade commercially available.

CO2 incubator (HH. CP-T80L, Yiheng Scientific Limited Company, China); Centrifuge (Eppendof-5415R); Nikon fluorescence microscope (E600); Enzyme linked immunoassay detector (DG5031, Hua Dong Vacuum Tube Factory, China); Filter and Pump (Millipore Corporation); Flow Cytometry (EPICS ALTRA II)

B. Cell culture

HEK293 cells, a cell line of human embryonic kidney, were grown in RPMI-1640 supplemented with 10% fetal bovine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mML glutamine in a humidified incubator at 37 °C and 5% CO2. The cells were pre-incubated overnight, and the medium was replaced with fresh culture medium. Then the cells were subsequently analyzed for this study.

C. Experimental design

1) MTT assay

HEK293 cells were treated in the 96-well plate for 12 hours with different doses of formaldehyde which contained the concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μmol/L. Eight holes were set for each concentration and control groups were set for reset. After that, 20μL MTT solution (5ng/nL) was added into each hole and the cells could continue to cultivate in the CO2 incubator for 4 hours. The supernatant was carefully absorbed and then 150μL DMSO was added, with shaking on the swing bed for 10 minutes in order to make the MTT reductive production dissolved completely. Optical absorbance of the reaction was measured spectrophotometrically at 490 nm.

2) Flow cytometry

HEK293 Cells were treated for 12 hours with different doses of formaldehyde which contained the concentrations of 0，30，60，120，240μmol/L, and then washed in PBS. After resuspended with small amouts of PBS, cells were injected into the ethanol quickly which was precooling in -20 ℃, and then fixed overnight in -20 ℃. After that, the cells were washed in PBS again, treated with RNase A in 37℃ for 30 minutes, and then treated with PI in the dark for 30 minutes. After filtration with 400 meshes, the cell cycle could be detected by flow cytometry..

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III. RESULTS AND ANALYSIS

A. *The results measured by MTT assay*

The MTT assay results in figure 1 showed that low dose of formaldehyde did not inhibit cell growth, but could facilitate cell growth. When the concentration of formaldehyde was 60μmol/L, the promotion was the strongest; when the concentration exceeded 60μmol/L, the promotion began to diminish and the inhibition began to enhance; when the concentration reached 80μmol/L, the inhibition began. And with the promotion concentration, the inhibition became more and more strong. Accordingly, the concentrations of 30, 60, 120, 240μmol/L formaldehyde were chose to detect and analyze the cell cycle.

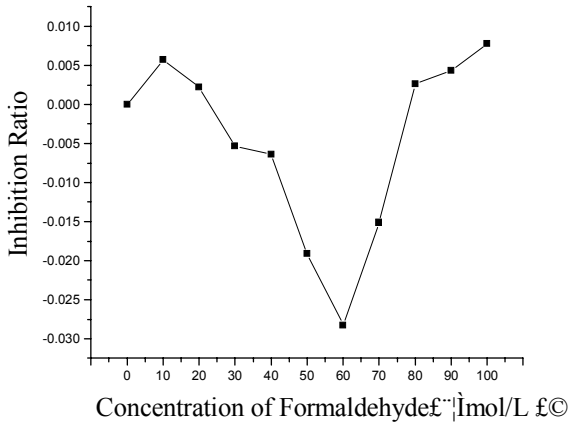
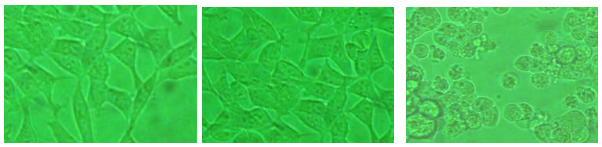


Figure 1. The influence of formaldehyde on the proliferation of HEK293 cells was detected by MTT assay

B. *The results measured by cell morphology*

After the HEK293 cells were treated for 12 hours, the inverted microscope was applied to detect the condition of cell growth and the digital camera was used to take pictures. Ii is clear from figure 2 that when the concentration of formaldehyde was 60μmol/L, the cell growth was in excellent condition and the cell density was much denser than control group. Whereas when the concentration was 240μmol/L, the cells assembled into groups and began shedding.

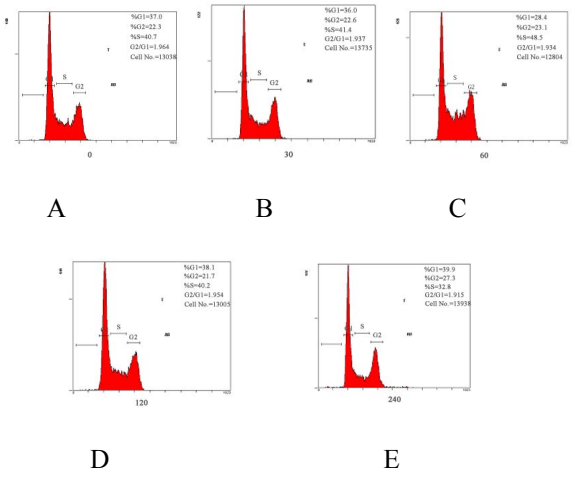


A: control group; B: 60 μ mol/L group; C: 240 μ mol/L group

Figure 2. After the HEK293 cells were treated for 12 hours, the condition of cell growth was detected by inverted microscope.

C. *The results measured by flow cytometry*

After the HEK293 cells were treated with different doses of formaldehyde for 12 hours, the flow cytometry was applied to detect the distribution of cell cycle (Figure 3, Table 1).



a: control group; b: 30μmol/L group; c: 60μmol/L group;

d: 120μmol/L group; e: 240μmol/L group

Figure 3. Example of a figure caption. (figure caption)

Table 1: The influence of formaldehyde on the distribution of cell cycle was detected by flow cytometry.

Groups (mol/L)	Distribution of Cell Cycle (%)		
	G1	S	G2
0	37.0	40.7	22.3
30	36.0	41.4	22.6
60	28.4	48.5	23.1
120	38.1	40.2	21.7
240	39.9	32.8	27.3

When the concentration of formaldehyde rose from 0μmol/L to 60μmol/L, the proportion of cells in phase G1 gradually decreased from 37% to 28%, whereas the proportion of cells in phase S gradually increased from 40.7% to 48%. This demonstrated that low dose of formaldehyde could induce the phase G1 cells into the S phase, thereby promoting cell proliferation. However, when the concentration of formaldehyde rose from 60μmol/L to 240μmol/L, the proportion of cells in phase G1, on the contrary, began to rise from 28.4% to 39.9%, whereas the proportion of cells in phase S began to decrease from 48.5% to 32.8%. This showed that high dose of formaldehyde could block the phase G1 cells into S phase and then inhibited the cell growth.

IV. Discussions

A. Formaldehyde and reactive oxygen species

Formaldehyde is a kind of small responsive and active molecule, which can cause the production of reactive oxygen species (ROS) and cause the increase of the oxidative stress through various channels in the body or cells. Firstly, formaldehyde can damage the organism or cell antioxidant systems, for example, the causation of the reduced activity of antioxidase which contains superoxide dismutase, peroxidase, and glutathione peroxidase etc, and the causation of the depletion of antioxidant substances such as reduced glutathione[1-3]. Secondly, as a free radical and an oxidizing agent, formaldehyde could generate strong oxidizing agents such as hydroxyl radical, with the similar effects of hydrogen peroxide[4]. Thirdly, formaldehyde could directly open the permeability transition pore of the mitochondrion, change the plasma membrane's ion permeability, degrade the mitochondrial membrane potential, inhibit the mitochondrial respiration, produce the ROS, and lead to oxidative stress[3]. The recent studies show that a modest increase of intracellular ROS levels can promote cell proliferation and differentiation; however, a significantly increase of the level of intracellular ROS is able to trigger cell apoptosis.

B. Endogenous formaldehyde

More and more evidences show that formaldehyde is not only an environmental toxicant, but also a normal metabolic intermediate in the organism[5]. There are various pathways for the formaldehyde generation. For example, after the N-methyl-, O-methyl-, S-methyl-complexes are catalyzed by cytochrome P450 or special peroxidase, and then are oxidative demethylated, formaldehyde can be generated. After the Semicarbazide-sensitive amine oxidase acts with the endogenous compounds such as aminoacetone and methylamine, formaldehyde can be produced[6]; With the help of the methyltransferases, pyridoxal phosphate catalyzed the conversion of glycine and serine can also generate formaldehyde[7].

C. Formaldehyde and cell proliferation

In this study, after the HEK293 cells were treated by the low dose of formaldehyde (60μmol/L) for 12 hours, the percentage of phase G1 is decreased, whereas the percentage of phase S is increased, which indicated that the DNA synthesis speed is promoted and FA would facilitate the proliferation of HEK293 cells; however, after the HEK293 cells were treated by the high dose of formaldehyde (240μmol/L) for 12 hours, the percentage of phase G1 is increased, whereas the percentage of phase S is decreased, which indicates that a large number of cells were blocked in phase G1 and can not enter phase S, thus inhibiting cell proliferation. At present, the mechanism of formaldehyde adjusts the cell proliferation is not yet clear. As an intracellular or intercellular messenger molecule, or changed the lever of intracellular GSH, formaldehyde may indirectly regulate the intracellular redox lever and then switch the signal protein's activity. The regulatory mechanism may be similar to the mechanism of the hydrogen peroxide's regulating cell proliferation, but the exact mechanism requires further experimental studies.

V. Conclusion

Formaldehyde can promote the cellular proliferation of the HEK293 cells at the low dose (60μmol/L) and can inhibit the cellular proliferation of the HEK293 cells at the high dose (240μmol/L).

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